FEBS 29692 FEBS Letters 579 (2005) 3807–3813

Identification of a villin-related tobacco protein as a novel cross-reactive plant allergen

Irene Mittermann[a,1], Viktor Voronin[b,2], Erwin Heberle-Bors[b,2], Rudolf Valenta[a,*]

[a] *Division of Immunopathology, Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria*
[b] *Department of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria*

Received 7 February 2005; revised 10 May 2005; accepted 25 May 2005

Available online 13 June 2005

Edited by Masayuki Miyasaka

Abstract In a paradigmatic approach we identified cross-reactive plant allergens for allergy diagnosis and treatment by screening of a tobacco leaf complementary DNA (cDNA) library with serum IgE from a polysensitized allergic patient. Two IgE-reactive cDNA clones were isolated which code for proteins with significant sequence similarity to the actin-binding protein, villin. Northern- and Western-blotting demonstrate expression of the villin-related allergens in pollen and somatic plant tissues. In addition, villin-related proteins were detected in several plant allergen sources (tree-, grass-, weed pollen, fruits, vegetables, nuts). A recombinant C-terminal fragment of the villin-related protein was expressed in *Escherichia coli*, purified and shown to react specifically with allergic patients IgE. After profilin, villin-related proteins represent another family of cytoskeletal proteins, which has been identified as cross-reactive plant allergens. They may be used for the diagnosis and treatment of patients suffering from multivalent plant allergies.

Keywords: Allergy; IgE; Diagnosis; Villin-related allergen; Cross-reactivity

1. Introduction

Pollen and plant food belong to the major environmental allergen sources causing allergic diseases [1]. IgE cross-reactivity to proteins that are present in various plants and plant tissues is a frequent cause of pollen and plant food allergy [2]. Cross-reactive plant allergens usually exhibit a high degree of sequence homology, occur in unrelated plant species and plant tissues (e.g., pollen, fruits, seeds, leaves), and contain cross-reactive IgE epitopes [3]. The spectrum of cross-reactive plant allergens responsible for pollen and plant food allergy comprises the cytoskeletal protein profilin [4,5], members of a family of proteins induced in plants after pathogen attack (i.e., pathogenesis-related plant proteins) and the family of lipid transfer proteins (LTP) [3]. Furthermore, pectin esterases [6], isoflavone reductases [7] and cyclophilins [8,9] have been identified recently as cross-reactive allergens in pollen and plant foods. Allergic cross-reactivity between pollen and plant food is mainly mediated by profilin and members of the pathogenesis-related plant proteins [3–5]. Among the members of the pathogenesis-related plant allergens, Bet v 1, the major birch pollen allergen has been extensively characterized [10]. Bet v 1-related allergens have been identified in apple (Mal d 1) [11], celery (Api g 1) [12], carrot (Dau c 1) [13], sweet cherry (Pru av 1) [14] and pear (Pyr c 1) [15] and also in asparagus, potato and hazelnut [16,17]. Allergic patients who are sensitized to Bet v 1 exhibit allergic reactions to pollens of birch-related trees (e.g., alder, hazel, hornbeam, oak) and to plant food, a combination of allergic symptoms termed oral allergy syndrome (OAS) [18]. Similarly, profilin-sensitized patients show allergic reactions to pollens of several unrelated plants (e.g., trees, grasses, weeds), vegetables, fruits, spices and nuts [5]. The three-dimensional structures of profilins [19] and Bet v 1-related allergens from several plants [14,20] have recently been determined and confirm that IgE cross-reactivity is due to structural similarities among these allergens.

Based on the fact that highly cross-reactive plant allergens occur also in plants or plant tissues which are not typical allergen sources, but are accessible for molecular cloning approaches [21], we have screened a tobacco (*Nicotiana tabacum*) leaf complementary DNA (cDNA) expression library with serum IgE from a patient with polysensitization to pollen and food from various plants. We report the isolation of IgE-reactive cDNA clones coding for villin-related proteins, a family of highly conserved cytoskeletal proteins. Furthermore, we provide evidence that villin-related allergens occur in pollen of trees, grasses, weeds, fruits, vegetables and nuts and thus may represent a novel family of cross-reactive allergens.

2. Materials and methods

2.1. Escherichia coli *strains, plasmids, patients sera, antibodies, allergen extracts, allergens*

Escherichia coli strain XL-1 Blue: *rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1 *lac*[F'proAB *lacI*^{q}ZΔEM15 Tn10(Tetr)]c and *E. coli* strain BL21 (DE): F$^-$, *omp*T $r_B^- m_B^-$ (DE) were obtained from Stratagene

*Corresponding author. Fax: +43 1 40400 5130.
E-mail addresses: irene.mittermann@meduniwien.ac.at (I. Mittermann), viktor.voronin@univie.ac.at (V. Voronin), erwin.heberle-bors@univie.ac.at (E. Heberle-Bors), rudolf.valenta@meduniwien.ac.at (R. Valenta).

[1] Fax: +43 1 40400 5130.
[2] Fax: +43 1 4277 9546.

Abbreviations: cDNA, complementary DNA; OAS, oral allergy syndrome; ORF, open reading frame; IPTG, isopropyl-β-thiogalactopyranoside; HSA, human serum albumin; LTP, lipid transfer protein; ntvln, villin-related protein from *Nicotiana tabacum*

0014-5793/$30.00
doi:10.1016/j.febslet.2005.05.066

(La Jolla, CA). Plasmid pET-23d used for expression of recombinant fragments of the villin-related proteins was purchased from Novagen (Madison, WI).

Patients allergic to pollen and plant-derived food (OAS) were characterized by IgE serology, positive case history and skin reactivity as described [22]. The specificity of IgE-reactivity of the villin-related allergen was confirmed with sera from patients with IgE against the villin-related protein (sera #1, 2) and with sera from patients allergic to other plant allergens (sera #3–6) in the dot-blot experiments. Mugwort (*Artemisia vulgaris*), timothy grass (*Phleum pratense*), birch (*Betula verrucosa*) and tobacco (*N. tabacum*) pollen were purchased from Allergon (Välinge, Sweden). Fresh fruits (apple) and vegetables (celery, carrot) were purchased at a local market. Tobacco leaves (cultivar Petit Havanna) were harvested directly into liquid nitrogen and stored at −70 °C until use. Recombinant Bet v 1 and Bet v 2 (birch profilin) were obtained from BIOMAY, Vienna, Austria (www.biomay.at).

2.2. Isolation and characterization of IgE-reactive clones from a tobacco leaf cDNA library

A tobacco (*N. tabacum* var. SR1) leaf expression cDNA library constructed in lambda ZAP (Stratagene) was screened with serum IgE from a patient with allergic polysensitization to pollen and plant food. Twohundredthousand plaque forming units of the library were screened for IgE-reactive clones as described [4]. Two IgE-reactive phage clones were purified to homogeneity and the corresponding plasmids pBSk+ were obtained by in vivo excision [23]. Plasmid DNA was prepared from the two clones using Quiagen tips (Quiagen, Hilden, Germany). The DNA sequence of both strands of each of the clones (clone ntvln1: 1917 bp, ORF: 1680 bp; clone ntvln2: 1999 bp, ORF: 1584 bp) was determined according to Sanger et al. [24] using ^{35}S dCTP (NEN, Stevenage, UK), a T7 sequencing kit (Pharmacia, Uppsala, Sweden), forward, reverse (Boehringer, Mannheim, Germany) and internal primers (MWG-Biotech AG, Ebersberg, Germany). The McVector program (Kodak Rochester, NY) was used to establish the deduced amino acid sequence of the open reading frame (ORF), to calculate p*I*, molecular weight, amino acid composition and secondary structure. The cDNA and deduced amino acid sequence of the isolated clones were compared with the sequences deposited in GenBank using the Blast program.

2.3. Northern-blot experiments

RNA was prepared from pollen and tobacco tissues according to Ausubel et al. [25]. Nitrocellulose membranes containing ≈20 μg RNA aliquots from mature pollen, leaves, stems, roots and seeds were hybridized with the full length ntvln1-derived cDNA as described [25]. The Northern-blot was probed with the constitutively expressed pCNT6 gene probe as described [26].

2.4. Expression and purification of the recombinant villin-related protein fragment; production of a specific rabbit antiserum

The cDNA coding for the longest villin-related protein fragment (ntvln1) was amplified by PCR, using the synthetic oligonucleotide primers (MWG-Biotech AG): Forward: 5′-CATGCCATGGAAGGGGGTGGAAAAATAGAGGTC-3′ and Reverse: 5′-CCG*CTCGAG*GAAGAGATCAACTTTCTTTTTGTG-3′. The primers contained an *Nco*I site (underlined) and an *Xho*I-site (italics), respectively, to allow in-frame insertion into plasmid pET-23d and the expression of a C-terminal hexahistidine-tagged villin-related fragment. The correct sequence of the expression construct was confirmed by double-strand DNA sequencing (MWG-Biotech AG).

The pET-23d-villin construct was transformed into *E. coli* BL21 (DE3) and grown in Luria Broth (LB) medium containing 100 mg/l ampicillin at 37 °C to an OD (600 nm) of 0.4. Protein expression was induced in liquid culture by addition of isopropyl-β-thiogalactopyranoside (IPTG) (0.5 mM) and the inclusion body fraction containing recombinant villin-related protein was prepared as described [27].

Recombinant His-tagged villin-related protein fragment was purified by Nickel affinity chromatography (Quiagen). The purity of the protein was checked by SDS–PAGE and Coomassie Blue staining [28] and its identity was confirmed with a monoclonal α-Histag antibody (Novagen). The protein concentration was determined by BCA assay (Pierce, Rockford, IL).

New Zealand white rabbits were immunized with purified recombinant villin-related protein using complete and incomplete Freund's adjuvant (Charles River, Kisslegg, Germany).

2.5. Specific IgE-reactivity of the recombinant villin-related protein fragment, cross-reactivity of specific antibodies

The specific IgE binding capacity of the purified villin-related protein was tested by immunoblotting [5] as well as by dot-blot assay under non-denaturing conditions. The dot blots contained 0.1 μg of recombinant villin-related protein, 0.1 μg of rBet v 1, rBet v 2 and 0.1 μg human serum albumin (HSA) (Behringwerke AG, Marburg, Germany). Nitrocellulose membranes were incubated with sera from allergic patients, and for control purposes, with serum from a non-allergic individual and bound IgE antibodies were detected with ^{125}I-labeled anti-human IgE antibodies (Pharmacia).

Rabbit antibodies raised against the villin-related protein and, for control purposes, the pre-immune serum (dilution 1:1000) were used to probe nitrocellulose-blotted pollen, fruit, vegetable, nut and tobacco leaf extracts. Bound rabbit antibodies were detected with a ^{125}I-labeled donkey anti-rabbit Ig antiserum (Amersham, Buckinghamshire, UK) as described [5]. Human IgE-reactivity to nitrocellulose-blotted plant extracts was studied as described for the dot-blot experiments.

2.6. IgG and IgE inhibition experiments

Rabbit antiserum (dilution 1:1000) raised against the villin-related protein and the corresponding pre-immune serum were incubated overnight with 50 μg of the villin-related protein [5]. Likewise serum from a patient, who exhibited IgE-reactivity to the villin-related protein in the dot-blot assay, was preabsorbed overnight with 10 μg of the recombinant villin-related protein [5]. All sera were also incubated with buffer without addition of the villin-related protein overnight at 4 °C.

Preabsorbed sera were then exposed to nitrocellulose-blotted tobacco pollen extract, and bound rabbit IgG or human IgE antibodies were detected as described above [5].

3. Results

3.1. IgE-antibodies of a patient with pollen and plant-food allergy identify cDNA clones coding for plant villin-related proteins

In a paradigmatic approach for the identification of highly cross-reactive plant allergens, serum IgE from a patient suffering from multivalent plant allergy (pollinosis, plant food allergy) and OAS was used to screen a cDNA library from tobacco leaves. Two independently isolated IgE-reactive clones (ntvln1, ntvln2) were purified to homogeneity by several rounds of screening. Sequence analysis showed that both clones exhibited a 86.96% sequence identity at the nucleotide level between each other (see EMBL database: ntvln1: AJ577852; ntvln2: AJ577853) and encoded polypeptides with significant sequence homology to villin (Fig. 1A).

The comparison of the deduced amino acid sequences of the two tobacco cDNAs (ntvln1, ntvln2) with the villins from lily (llvln), Arabidopsis (atvln1-3) and man (hsvln) is shown in Fig. 1A. Villins are ubiquitous actin-binding proteins with a characteristic structure consisting of 7 domains, six gelsolin repeat domains and one headpiece domain. Domain 2 binds phosphoinositides, domains 1–6 are involved in actin-binding and the C-terminal headpiece domain binds actin even as an isolated domain (Fig. 1B) (www.bms.ed.ac.uk/research/others/smaciver/Cyto-Topics/GSVilStruct.htm) [29]. Both clones code for C-terminal fragments of villin-related protein isoforms comprising domains 4–6, the linker piece and the C-terminal headpiece. A molecular weight of 61.9 kDa and an isoelectric point of 5.32 were calculated for the first tobacco IgE-reactive

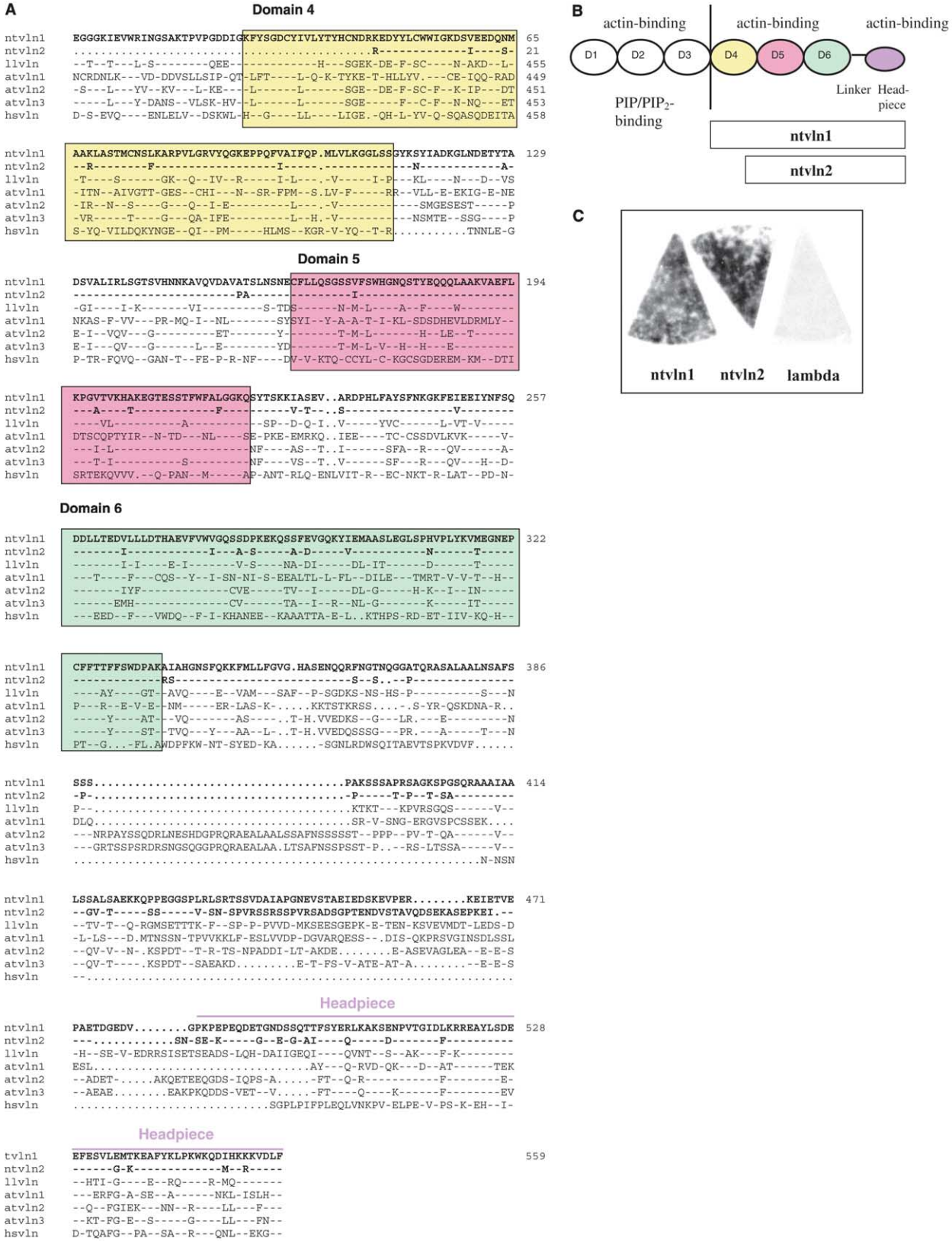

Fig. 1. Characterization of IgE-reactive tobacco villin-related isoforms. (A) Amino acid sequences (single letter code) of two IgE-reactive *N. tabacum* villin-related protein fragments (ntvln1, ntvln2) were aligned with villins from *Lilium longiflorum* (llvln [AF088901]), *Arabidopsis thaliana* (atvln1 [AF081201], atvln2 [AF081202], atvln3 [AF081203]) and *Homo sapiens* (hsvln [NM_007127]). The domains and headpieces of the proteins are coloured. Identical amino acids are indicated by dashes, points were introduced to bridge gaps. The cDNA and deduced amino acid sequences of the two tobacco villin-related clones (ntvln1,2) have been submitted to the EMBL database under the Accession Nos. ntvln1: AJ577852 and ntvln 2: AJ577853. (B) General structure of villin. Domains, linker and headpiece are shown. PIP/PIP2- and actin-binding sites are indicated. (C) Specific reactivity of nitrocellulose-bound phage expressing ntvln1 and ntvln2 vs. empty phage with serum IgE from a patient.

protein (ntvln1). The cDNA of the second clone codes for an IgE-reactive protein of 57.8 kDa with a predicted isoelectric point of 5.59 (ntvln2). The tobacco villin-related proteins share the highest degree of sequence identity with villins from lily (68%), Arabidopsis (atvln1 38.5%, atvln2 64.7%, atvln3 65.3%) and also exhibit significant sequence identity with human villin (34.4%). Nitrocellulose-bound phage expressing ntvln1 and ntvln2 cross-reacted with serum IgE from an allergic patient whereas no IgE-reactivity to empty phage was observed (Fig. 1C).

3.2. Plant villin-related proteins occur as isoallergens which are expressed in pollen and somatic plant tissues

Differences in the cDNA and amino acid sequences of the two villin-related clones indicate to the occurrence of isoforms. This assumption was supported by Southern-blot experiments showing that the villin-related cDNA hybridized with several bands in digested genomic tobacco DNA (data not shown). Several important cross-reactive plant allergens implicated in pollen-food cross-reactivity (e.g., Bet v 1, profilin) are preferentially expressed in pollen and to a much lower degree in somatic plant tissues (e.g., fruits, vegetables) [21,30]. Hybridization of the complete villin-related cDNA of ntvln1 with RNA from mature pollen, young leaves, old leaves, stems and roots detected villin transcripts of ≈4.7 kb and more weakly at 3 kb (marked with asterisks) mainly in young somatic tissues (i.e., young leaves), followed by pollen, old leaves and seeds (data not shown) and, in very low amounts in stems (Fig. 2). Hybridization of the same membrane with the constitutively expressed pCNT6 gene probe showed that comparable amounts of intact RNA had been transferred to the membrane.

3.3. Expression and purification of an IgE-reactive recombinant tobacco villin-related protein fragment

When tested for IgE-reactivity in a plaque-assay both recombinant villin-related protein fragments (ntvln1, ntvln2) showed comparable IgE-reactivity (Fig. 1C). We therefore expressed the longer fragment (ntvln1) as C-terminally hexahistidine-tagged protein in *E. coli*. The recombinant villin-related

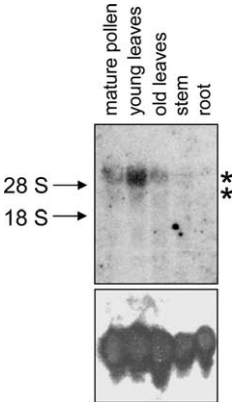

Fig. 2. Detection of villin-related transcripts in different tobacco tissues. Twenty micrograms of nitrocellulose-blotted RNA from mature pollen, young and old leaves, stems and roots was hybridized with a villin-related cDNA probe. The positions of the 18S and 28S rRNA (arrows) as well as of the villin-related transcripts (asterisks) are indicated. Lower part: The same membrane hybridized with the constitutively expressed pCNT6 gene.

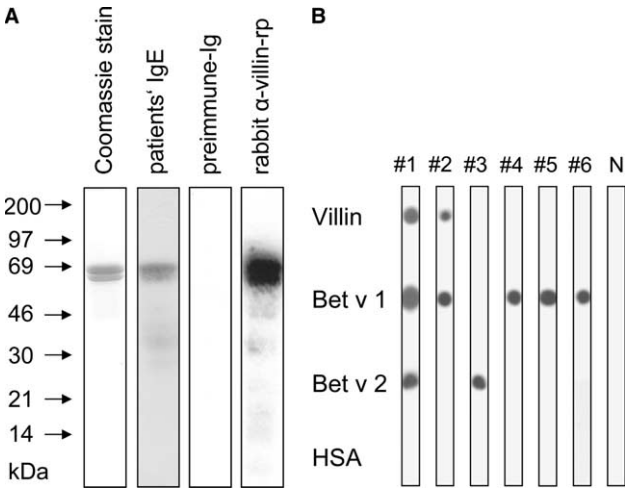

Fig. 3. Purification and antibody reactivity of the purified recombinant tobacco villin-related protein fragment. (A) Coomassie blue-stained SDS–PAGE containing purified recombinant villin-related protein. Approximately 5 μg/lane of nitrocellulose-blotted purified recombinant villin was exposed to allergic patients IgE, rabbit IgG raised against the recombinant villin-related protein or the rabbit pre-immune IgG. (B) Specific reactivity of recombinant villin-related protein with allergic patients IgE antibodies. Nitrocellulose-dotted recombinant villin-related protein, rBet v 1, rBet v 2 and HSA (each 0.1 μg/dot) were probed with sera from plant allergic patients (#1–6) and a non-allergic individual (N). Bound IgE antibodies were detected with ^{125}I-labeled anti-human IgE antibodies.

protein fragment accumulated in the inclusion body fraction of *E. coli* and was purified using nickel affinity chromatography (typical yield: 5–6 mg/l culture) (Fig. 3A). It migrated as a 66–68 kDa band in SDS–PAGE, reacted with IgE from sensitized allergic patients as well as with a rabbit antiserum-raised against the purified protein but not with the pre-immune serum (Fig. 3A). The specific IgE-reactivity of the recombinant villin-related protein fragment was confirmed by dot-blot experiments. As exemplified in Fig. 3B, we found that sera from OAS patients with allergy to pollen and plant food showed IgE-reactivity to the villin-related fragment (#1, 2). Certain sera (e.g., #1, 3) reacted also with another highly cross-reactive plant allergen, profilin (i.e., Bet v 2) which is also a cytoskeletal protein. Sera from patients with OAS due to exclusive reactivity to the major birch pollen allergen Bet v 1 (#4, 5, 6) did not show IgE-reactivity to the villin-related protein confirming the specificity of IgE reactivities. Serum from a non-atopic person did not react with the purified proteins (Fig. 3B: lane N). None of the sera displayed IgE-reactivity with HSA (negative control).

3.4. Sera from patients with IgE to the recombinant villin-related protein and rabbit antibodies raised against the recombinant villin-related fragment detect villin-like proteins in common plant allergen sources

In order to study whether villin-related proteins can be detected in important plant allergen sources a rabbit antiserum was raised against the recombinant villin-related fragment (α-villin-rp). Compared to the pre-immune serum, it reacted specifically with bands of ≈50 kDa in pollen extracts from tobacco, timothy grass, mugwort and birch (Fig. 4A and B). A specific immune-reactive band of 50 kDa was also detected in apple, carrot and celery (Fig. 4A and B). In walnut and peanut

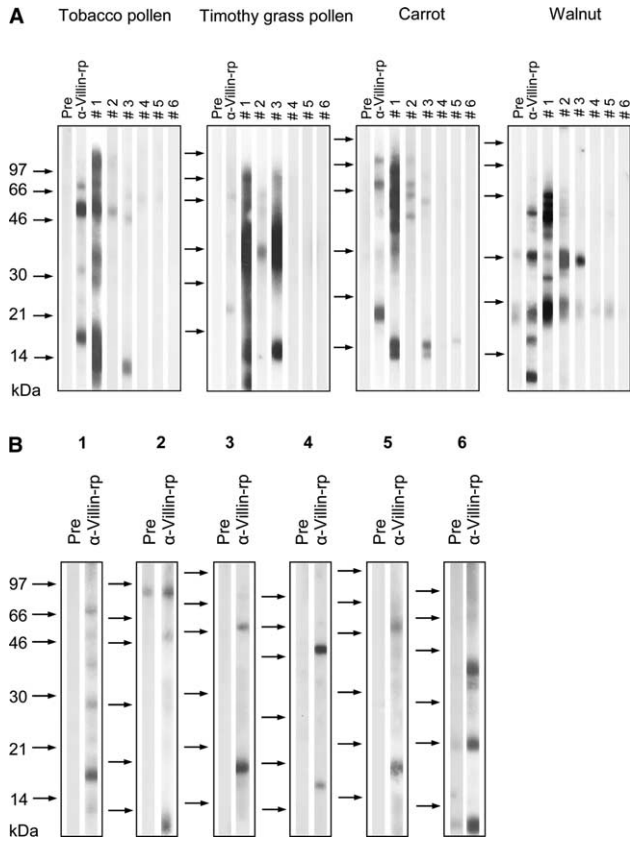

Fig. 4. Presence of villin-related proteins in important plant allergen sources. Seventy micrograms/lane of nitrocellulose-blotted plant extracts (A: tobacco pollen, timothy grass pollen, carrot, walnut) were probed with rabbit antibodies specific for the recombinant villin-related protein (α-villin-rp), the corresponding pre-immune serum (pre) and with the sera used in Fig. 3B. Additional blotted plant extracts (B: tobacco leaf, 1; mugwort pollen, 2; birch pollen, 3; celery, 4; apple, 5; peanut, 6) were probed with rabbit antibodies specific for the recombinant villin-related protein (α-villin-rp) and the corresponding pre-immune serum (pre).

extracts, rabbit antibodies raised against the villin-related protein reacted specifically with bands between 14 and 40 kDa (Fig. 4A and B). The specificity of the reactivity of the rabbit antiserum raised against the recombinant villin-related fragment (α-villin-rp) was confirmed by immunoblot inhibition studies (Fig. 5). Preabsorption of the antiserum with the recombinant villin-related protein (Fig. 5, lane α-villin-rp +) led to a complete loss of IgG binding to several bands in the nitrocellulose-blotted tobacco pollen extract.

Sera from patients with specific IgE antibodies against the villin-related protein (#1, 2) showed IgE-reactivity to proteins of comparable molecular weight (of 50 kDa) as those detected by the rabbit antibodies in tobacco pollen, timothy grass pollen, carrot and walnut, but also to other allergens. Serum from the Bet v 2 (#3) and the Bet v 1 (#4–6) allergic patients did not show IgE-reactivity to the bands defined by the rabbit antiserum raised against the villin-related protein in the pollen and plant food extracts (Fig. 4A). Preabsorption of serum from a patient containing IgE against the villin-related protein with the recombinant villin-related protein (Fig. 5, lane patient +), but not with buffer alone (Fig. 5, lane patient -) led to a reduction of IgE binding to a band of ≈50 kDa in nitrocellulose-blotted tobacco pollen extract.

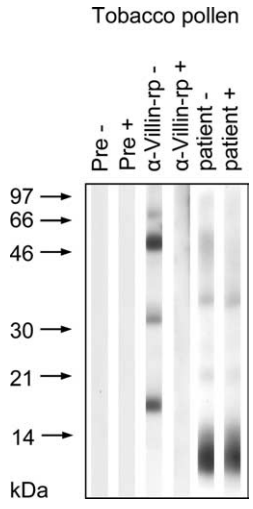

Fig. 5. Identification of villin-related antigens in tobacco pollen by inhibition experiments. Strips of nitrocellulose containing blotted tobacco pollen extract were exposed to rabbit IgG raised against the recombinant villin-related protein (α-villin-rp), to the rabbit pre-immune IgG (pre) or to sera from an allergic patient (patient) that has been preadsorbed without (lanes −) or with (lanes +) recombinant villin-related protein.

4. Discussion

In this study we have identified proteins related to the highly conserved actin-binding protein villin, as allergens that are present in pollen from trees, grasses and weeds as well as in plant-derived food. Such as profilins, that are highly cross-reactive plant allergens [4,5], villin-related proteins are cytoskeletal proteins. Based on the assumption, that highly cross-reactive plant allergens are also expressed as evolutionary conserved proteins in plants and plant tissues that are not regarded as typical allergen sources, a tobacco leaf library was screened with serum IgE from a polysensitized allergic patient. Two IgE-reactive cDNA clones were isolated and identified by sequence comparisons as isoforms coding for villin-related proteins.

The villin/gelsolin protein family of actin-binding proteins [31] is modulated by calcium and polyphosphoinositides [32]. Villins typically consist of six highly conserved gelsolin-repeat domains which are connected via a less conserved linker sequence with a C-terminal headpiece domain (www.bms.ed.ac.uk/research/others/smaciver/Cyto-Topics/Villin_family.htm) [29]. The fact that patients IgE antibodies independently identified two cDNA clones coding for villin-related protein fragments comprising three of the six actin-binding domains indicates that the C-terminal portions of the villin-related proteins are important for IgE recognition. Several attempts to isolate IgE-reactive cDNA clones coding for isoforms containing additional actin-binding domains in their N-terminus failed. We also could not isolate longer cDNA clones coding for villin-related proteins using synthetic oligonucleotides for screening of the original library. It is therefore possible that the tobacco villin-related isoforms represent shorter forms of the villin-like proteins, which may have evolved by less extensive gene duplication of the actin-binding domains compared to villins. The latter assumption is supported by the observation that the rabbit antiserum raised against the purified recombinant villin-related protein reacted with bands of ≈50 kDa in pollens of trees, grasses and weeds and primarily

identified bands of less than 80 kDa in tobacco leaf as well as in fruit, nut and vegetable extracts. The 50 kDa bands detected by the rabbit antiserum raised against the recombinant villin-like protein are specific because the pre-immune serum did not react with these bands. Moreover, it could be demonstrated that pre-incubation of the rabbit antiserum with the recombinant villin-related protein completely inhibited IgG binding to these bands in blotted tobacco pollen extract. The 50 kDa band most likely represents a degradation product of the villin-related protein such as the low molecular bands (e.g., 18–19 kDa bands) detected by the antiserum in tobacco leaf, birch pollen, apple and carrot extracts.

On the other hand, Northern-blot experiments revealed hybridization of the cDNA coding for the villin-related protein with transcripts of more than 3 kb in pollen and leaf tissues similar as has been demonstrated for other plant villins [33] and it is therefore possible that tobacco also contains larger villin-related protein isoforms.

The longer recombinant villin-related fragment (ntvln1) was purified and shown to react specifically with serum IgE from plant-allergic patients suffering from OAS. Furthermore, it induced histamine release from basophils of a sensitized patient (data not shown). A rabbit antiserum which was raised against the purified recombinant villin-related protein showed specific reactivity to antigens in weed-, grass- and tree pollens as well as in fruit-, vegetable- and nut extracts. These results indicate that villin-related proteins represent cross-reactive allergens in pollens and plant food. The finding that a villin-like allergen (i.e., gelsolin) has recently been described in the house dust mite [34] raises even the possibility that villin-related proteins might represent cross-reactive allergens even outside the plant kingdom.

Acknowledgements: This study was supported by Grants Y078GEN of the Austrian Science Fund, by CeMM-Center of Molecular Medicine, a basic research institute within the companies of the Austrian Academy of Sciences and by a research grant from Pharmacia Diagnostics, Uppsala, Sweden.

References

[1] Kay, A.B. (1997) Allergy and Allergic Diseases, Blackwell Science, Oxford.
[2] Bohle, B., Swoboda, I., Spitzauer, S., Breiteneder, H., Hoffmann-Sommergruber, K., Scheiner, O., Ebner, C., Kraft, D. and Valenta, R. (2003) in: Food Allergy: Adverse Reactions to Foods and Food Additives (Metcalfe, D.D., Sampson, H.A. and Simon, R.A., Eds.), 3rd edn, pp. 38–50, Blackwell Science, Oxford.
[3] Breiteneder, H. and Ebner, C. (2000) Molecular and biochemical classification of plant-derived food allergens. J. Allergy Clin. Immunol. 106, 27–36.
[4] Valenta, R., Duchene, M., Pettenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D. and Scheiner, O. (1991) Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. Science 253, 557–560.
[5] Valenta, R., Duchene, M., Ebner, C., Valent, P., Sillaber, C., Deviller, P., Ferreira, F., Tejkl, M., Edelmann, H., Kraft, D. and Scheiner, O. (1992) Profilins constitute a novel family of functional plant pan-allergens. J. Exp. Med. 175, 377–385.
[6] Mahler, V., Fischer, S., Heiss, S., Duchene, M., Kraft, D. and Valenta, R. (2001) cDNA cloning and characterization of a cross-reactive birch pollen allergen: identification as a pectin esterase. Int. Arch. Allergy Immunol. 124, 64–66.
[7] Karamloo, F., Schmitz, N., Scheurer, S., Foetisch, K., Hoffmann, A., Haustein, D. and Vieths, S. (1999) Molecular cloning and characterization of a birch pollen minor allergen, Bet v 5, belonging to a family of isoflavone reductase-related proteins. J. Allergy Clin. Immunol. 104, 991–999.
[8] Cadot, P., Diaz, J.F., Proost, P., Van Damme, J., Engelborghs, Y., Stevens, E.A.M. and Ceuppens, J.L. (2000) Purification and characterization of an 18-kd allergen of birch (*Betula verrucosa*) pollen: identification as a cyclophilin. J. Allergy Clin. Immunol. 105, 286–291.
[9] Flueckiger, S., Fijten, H., Whitley, P., Blaser, K. and Crameri, R. (2002) Cyclophilins, a new family of cross-reactive allergens. Eur. J. Immunol. 32, 10–17.
[10] Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O. and Breitenbach, M. (1989) The gene coding for the major birch pollen allergen Bet v 1, is highly homologous to a pea disease resistance response gene. EMBO J. 8, 1935–1938.
[11] Vanek-Krebitz, M., Hoffmann-Sommergruber, K., Laimer-da-Camara-Machado, M., Susani, M., Ebner, C., Kraft, D., Scheiner, O. and Breiteneder, H. (1995) Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. Biochem. Biophys. Res. Commun. 214, 538–551.
[12] Breiteneder, H., Hoffmann-Sommergruber, K., O'Riordain, G., Susani, M., Ahorn, H., Ebner, C., Kraft, D. and Scheiner, O. (1995) Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. Eur. J. Biochem. 233, 484–489.
[13] Hoffmann-Sommergruber, K., O'Riordain, G., Ahorn, H., Ebner, C., Laimer-da-Camara-Machado, M., Pühringer, H., Scheiner, O. and Breiteneder, H. (1999) Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. Clin. Exp. Allergy 29, 840–847.
[14] Neudecker, P., Schweimer, K., Nerkamp, J., Scheurer, S., Vieths, S., Sticht, H. and Rosch, P. (2001) Allergic cross-reactivity made visible: solution structure of the major cherry allergen Pru av 1. J. Biol. Chem. 276, 22756–22763.
[15] Karamloo, F., Scheurer, S., Wangorsch, A., May, S., Haustein, D. and Vieths, S. (2001) Pyr c 1, the major allergen from pear (*Pyrus communis*), is a new member of the Bet v 1 allergen family. J. Chromatogr. B. Biomed. Sci. Appl. 25, 281–293.
[16] Warner, S.A., Scott, R. and Draper, J. (1992) Characterisation of a wound-induced transcript from the monocot asparagus that shares similarity with a class of intracellular pathogenesis-related (PR) proteins. Plant Mol. Biol. 19, 555–561.
[17] Ebner, C., Hirschwehr, R., Bauer, L., Breiteneder, H., Valenta, R., Ebner, H., Kraft, D. and Scheiner, O. (1995) Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J. Allergy Clin. Immunol. 95, 962–969.
[18] Ortolani, C., Ispano, M., Pastorello, E.A., Ansaloni, R. and Magri, G.C. (1989) Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. J. Allergy Clin. Immunol. 83, 683–690.
[19] Fedorov, A.A., Ball, T., Mahoney, N.M., Valenta, R. and Almo, S.C. (1997) The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin. Structure 15, 33–45.
[20] Gajhede, M., Osmark, P., Poulsen, F.M., Ipsen, H., Larsen, J.N., Joost van Neerven, R.J., Schou, C., Lowenstein, H. and Spangfort, M.D. (1996) X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. Nat. Struct. Biol. 3, 1040–1045.
[21] Mittermann, I., Swoboda, I., Pierson, E., Eller, N., Kraft, D., Valenta, R. and Heberle-Bors, E. (1995) Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation. Plant Mol. Biol. 27, 137–146.
[22] Kazemi-Shirazi, L., Pauli, G., Purohit, A., Spitzauer, S., Fröschl, R., Hoffmann-Sommergruber, K., Breiteneder, H., Scheiner, O., Kraft, D. and Valenta, R. (2000) Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many

forms of plant food allergy. J. Allergy Clin. Immunol. 105, 116–125.
[23] Short, J.M., Fernandez, J.M., Sorge, J.A. and Huse, W.D. (1988) Lambda ZAP: a bacteriophage lambda expression vector with in vivo excision properties. Nucleic Acids Res. 16, 7583–7600.
[24] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[25] Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J. and Struhl, K. (1987) Current Protocols in Molecular Biology, Wiley, New York, pp. 4.9.1–4.9.5.
[26] Memelink, J., Hoge, J.H.C. and Schilperoort, R.A. (1987) Cytokinin stress changes the developmental regulation of several defence-related genes in tobacco. EMBO J. 6, 3579–3583.
[27] Vrtala, S., Susani, M., Sperr, W.R., Valent, P., Laffer, S., Dolecek, C., Kraft, D. and Valenta, R. (1996) Immunologic characterization of purified recombinant timothy grass pollen (*Phleum pratense*) allergens (Phl p 1, Phl p 2, Phl p 5). J. Allergy Clin. Immunol. 97, 781–787.
[28] Fling, S.P. and Gregerson, D.S. (1986) Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea. Anal. Biochem. 155, 83–88.
[29] Vardar, D., Chishti, A.H., Frank, B.S., Luna, E.J., Noegel, A.A., Oh, S.W., Schleicher, M. and McKnight, C.J. (2002) Villin-type headpiece domains show a wide range of F-actin-binding affinities. Cell Motility Cytoskeleton 52, 9–21.
[30] Pettenburger, K., Tejkl, M., Valenta, R., Hoffmann-Sommergruber, K., Swoboda, I., Heberle-Bors, E., Breitenbach, M., Rumpold, H., Kraft, D. and Scheiner, O. (1992) Distribution of allergens and allergen-coding mRNAs in various tissues of white birch. Mol. Immunol. 29, 1401–1406.
[31] Andre, E., Lottspeich, F., Schleicher, M. and Noegel, E. (1988) Severin, gelsolin, and villin share a homologous sequence in regions presumed to contain F-actin severing domains. J. Biol. Chem. 263, 722–727.
[32] Janmey, P.A., Lamb, J., Allen, P.G. and Matsudaira, P.T. (1992) Phosphoinositide-binding peptides derived from the sequences of gelsolin and villin. J. Biol. Chem. 267, 11818–11823.
[33] Vidali, L., Yokota, E., Cheung, A.Y., Shimmen, T. and Hepler, P.K. (1999) The 135 kDa actin-bundling protein from *Lilium longiflorum* pollen is the plant homologue of villin. Protoplasma 209, 283–291.
[34] Kawamoto, S., Suzuki, T., Aki, T., Katsutani, T., Tsuboi, S., Shigeta, S. and Ono, K. (2002) Der f 16: a novel gelsolin-related molecule identified as an allergen from the house dust mite, *Dermatophagoides farinae*. FEBS Lett. 516, 234–238.